Swimming depth and behaviour of newly recruiting post-larvae of *Sicyopterus japonicus* (Gobioidei: Sicydiinae) in the estuary of the Ota River, Wakayama, Japan

by

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Abstract. – Recruitment from the sea to freshwater is a turning point in the life history of amphidromous fish. The amphidromous gobioid subfamily Sicydiinae is distributed widely in Indo-Pacific regions. To understand their ecology at recruitment, which is poorly known for this subfamily, underwater observations of recruiting post-larvae of *Sicyopterus japonicus* were conducted and their swimming depth was measured in the estuary of the Ota River of Wakayama, Japan. During 16 observation periods from April to July in 2006, 2007 and 2008, newly recruiting post-larvae were observed swimming in shoals in the middle layer of the lower half of the water column near the river banks in the upper area of study site and in the upper half of the water column near the river mouth. Although the estuary appears to be a temporary transit area for newly recruiting post-larvae, it may be an important place for them to adapt to their later benthic herbivorous life in freshwater.

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Résumé. – Profondeur de nage et comportement des post-larves de *Sicyopterus japonicus* (Gobioidei: Sicydiinae) lors de leur recrutement dans l'estuaire de la rivière Ota, Wakayama, Japon.

Le recrutement en eau douce des larves venant de la mer est une étape clé du cycle de vie des poissons amphidromes. La sous-famille de gobies amphidromes, les Sicydiinae, est distribuée à travers tout l'Indo-Pacifique. Pour comprendre leur comportement lors de leur recrutement, qui est peu connu pour cette sous-famille, on a observé sous l'eau, dans l'estuaire de la rivière Ota à Wakayama au Japon, des post-larves de *Sicyopterus japonicus* et leur profondeur de nage a été mesurée. Au cours de 16 périodes d'observation entre avril et juillet en 2006, 2007 et 2008, les post-larves ont été observées, nageant en bancs dans la moitié inférieure de la colonne d'eau, près des berges de la rivière. Bien que l'estuaire semble être un lieu de transition pour les post-larves nouvellement recrutées, c'est également un endroit important pour qu'elles s'adaptent à leur prochain mode de vie en tant qu'herbivore benthique en eau douce.

Key words
Gobioidei
Sicydiinae
Sicyopterus japonicus
Japan
Ota River
Amphidromy
Swimming depth
Behaviour

An amphidromous life history includes spawning in freshwater, with larvae drifting downstream to the sea where they have an oceanic larval life before migrating back into the rivers to grow and reproduce (McDowall, 1988). Amphidromous species of fish, molluscs and crustaceans are abundant in tropical and subtropical oceanic islands in Indo-Pacific regions as well as in temperate regions such as Japan and New Zealand (Keith, 2003; Watanabe *et al*., 2014). For amphidromous fish, recruitment from the sea to freshwater is a turning point of their life-cycling requiring metamorphosis to adapt their physiology, behaviour and even morphology to a different environment and different feeding habits.

The amphidromous goby *Sicyopterus japonicus* (Tanaka, 1909) (Gobioidei: Sicydiinae) inhabits the subtropical and temperate regions in East Asia from Taiwan to Japan, and is the only species distributed in the temperate region among this subfamily (Keith *et al*., 2015). Recently, several of their life history traits have been studied (Iida *et al*., 2009, 2010, 2013; Yamasaki *et al*., 2011) and their recruitment has been analysed (Iida *et al*., 2008, 2015). They recruit into rivers as post-larvae and metamorphose into juveniles. Juveniles of *S. japonicus* have an inferior mouth and almost complete pigmentation while post-larvae have forward protruding mouth as most fish larvae do (*S. lagocephalus* in Keith *et al*., 2008 and *S. japonicus* in Iida *et al*., 2015). They are reported to recruit at a constant size range (approximately 27 mm in standard length: SL) and have a unique head-down stationary holding (hovering) behaviour when they enter the river (Fig. 1B; Iida *et al*., 2015), but other details of their ecology during recruitment are still unknown despite the

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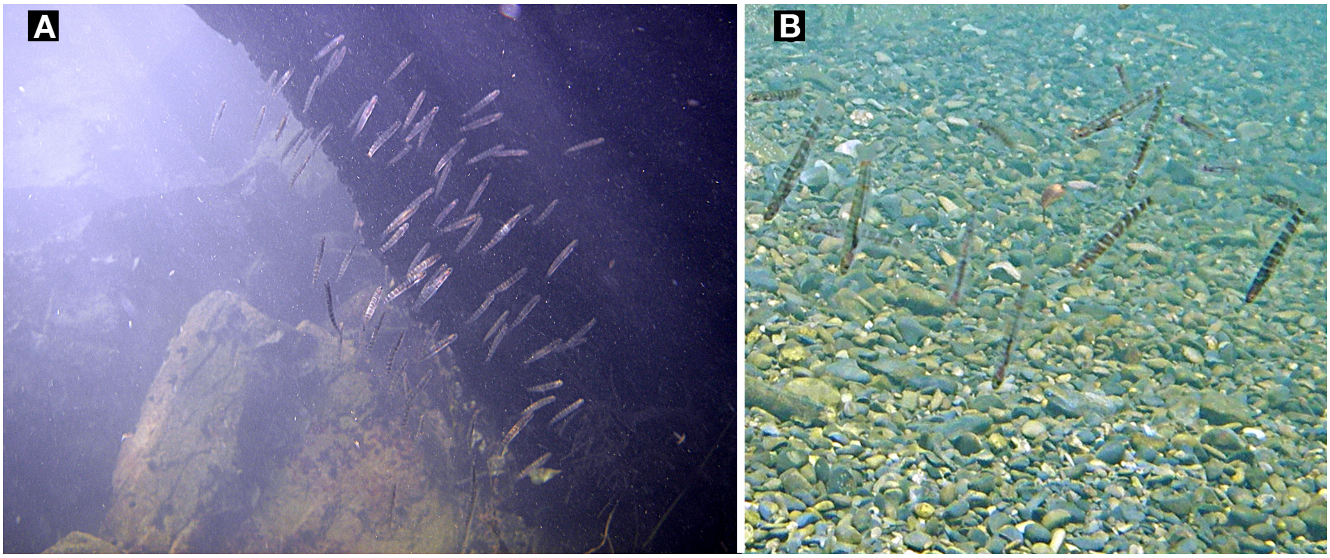

Figure 1. – **A**: A shoal of newly recruiting post-larvae of *Sicyopterus japonicus* swimming near rocks along the river bank; **B**: Head-down stationary holding (hovering) behaviour in the estuary of the Ota River.

likely importance of the transition from the sea to the river. Where they reside within the water column in the ocean is still unknown (Watanabe *et al.*, 2011), though it would be expected to be at shallow depths near the surface. Understanding their swimming depth and behaviour at recruitment in the estuary would be also useful to infer where they reside in the sea since newly recruiting post-larvae were in the sea just before entering the river and might retain ecological characteristics from their life in the sea. The objective of this study is to help understanding their swimming behaviour at recruitment with special reference to their swimming depth as they enter the river mouth and estuary.

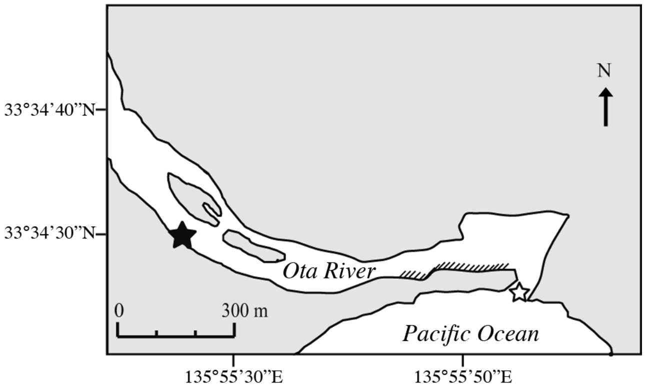

Figure 2. – Map of the study area the estuary of the Ota River, Wakayama, Japan with the closed and opened (river mouth) stars showing starting and end points of underwater observation. Observations were not conducted in the shaded area on the right side because larvae avoided those areas.

MATERIALS AND METHODS

Characterisation of the study area

The study was conducted in the lower reaches and estuary of the Ota River in Wakayama Prefecture of Japan (33˚34'N, 135˚55'E; Fig. 2). Observations were done in the area from the river mouth to 0.9 km upstream where there is a salinity influence depending on tidal changes, and where many newly recruiting post-larvae of *Sicyopterus japonicus* could be seen during our previous studies (Iida *et al.*, 2008, 2015). The river width in the area ranged from about 20 to 80 m (see details of the study area in Iida *et al.*, 2015). The salinity of surface water at the uppermost area of this study ranged from 0 to 27 and was mainly 0 depending on tidal changes and river water discharge (Iida, unpubl. data). Salinity was measured once at an area 5 m deep at the river mouth at every 1 m depth on 25 July 2008 and stratification was detected (salinity: 30, 31, 28, 31, 15 and 9, from bottom to the surface) with the freshwater remaining at the surface. To obtain basic information about the flow speed of the river, water velocity at the uppermost area of this study was measured by a current meter (VR-301, Kenek Inc.) on 18 May 2007. The river width was 56 m and measurements were made at 1.5, 11.5, 21.5, 31.5, 41.5 and 51.5 m from the right river bank. Water current was fast at the centre and slow near the banks: 1.5 m from the right river bank: 5.3 cm/sec, 11.5: 16.4, 21.5: 26.5, 31.5: 25.9, 41.5: 15.3, 51.5: 4.6. Depending on the flow volume of the river, and ebb and flood tides, the water depths in the observation area ranged approximately from 3 to 5 m at the river mouth to 30 m upstream, and 35-120 cm in the upper area. Substratum in the observation area was mainly cobble in the centre of the flow and sandy substrates near the banks. Vegetation was present on the sandbars and right banks except from 50 to 300 m upstream of the river mouth. On the left river bank, rocks and concrete tetrapods were placed along the river for erosion control.

Observation methodology

The snorkelling observations were done in 2006, 2007 and 2008 during daytime mainly at the beginning of a flood tide when the larvae enter the river (Iida *et al.*, 2015) by one observer (M. Iida) and started from the upper area (0.9 km from the river mouth) and ended at the river mouth with a zigzag swimming between right and left banks. Tidal changes were checked by a tide table. Duration of observations were 30-70 min each. In the right river side area, about 50 to 300 m from the river mouth, larvae were not seen in preliminary observations, probably because of no vegetation and rocks where larvae were often observed, thus observations were not conducted in that area (Fig. 2). As observations in 2006 and 2007 were preliminary, only the number of larvae in shoals were counted. In 2008 we also counted the larvae within shoals, and measured swimming depths of larvae relative to the surface and water depth of the river at the observation locations. When post-larval *S. japonicus* were observed, a 1 m stick with marks at every 20 cm was used to measure their swimming depth. As water depth was greater than 1 m near the river mouth, a rope with marks at every 50 cm attached to a float and a weight was used for measuring the depth of the shoal relative to the surface. When post-larvae were observed in a shoal, the shallowest and deepest depths of the shoal were measured and a central value was calculated for later analysis. As water depth varied, relative values were used instead of absolute ones. During their recruitment periods (Iida *et al.*, 2015), the snorkelling observations were conducted a total of 16 times: 5 and 20 May in 2006, 29 April, 18 and 22 May, 2 and 7 June in 2007, and 26 April, 9 and 23 May, 7, 17 and 18 June, 2, 7 and 12 July in 2008. The observations were done on sunny days with high water clarity for visual observations.

RESULTS

A total of more than 50 shoals of newly recruiting *S. japonicus* post-larvae were observed during the three years. At least one post-larva was observed on all days of observations from April to July. In 2008 when there were frequent observations (9), the numbers of larvae were more in May (n = 7-190 in each shoal) and June (n = 2-500) and lower in April (n = 8-15) and July (2-20). The number of newly recruiting post-larvae observed varied from a single individual to a shoal of about 500 individuals, with the shoals mainly being composed of 20-50 individuals. A total of 10 single post-larvae and 31 shoals of about 2-500 individuals were observed in 2008 that were included in the analysis of swimming depth. The largest shoal of about 500 individuals was seen on 7 June 2008 near the river mouth. The shoals were composed either of only *S. japonicus* or a combination of newly recruiting post-larvae including other amphidromous gobioids of *Gymnogobius breunigii*, *G. petschiliensis*, and *Rhinogobius* spp., and juvenile/adult estuarine gobies *Parioglossus dotui* that had similar body sizes as *S. japonicus*. Several *Rhinogobius* species (*R. similis* formally known as *R. giurinus*; *R. nagoyae*: *R.* sp. CB; *R. fulviatilis*: *R.* sp. LD; and *R.* sp. CO) are in the Ota River (Iida *et al.*, unpubl. data) but their post-larvae are similar in morphology. Thus we did not identify them into species during underwater observations. Ratios of *S. japonicus* and other species varied from a single larva of other species to almost all being other species. Among 10 single post-larvae and 31 shoals observed in 2008, six single and 23 shoals were composed of only *S. japonicus*.

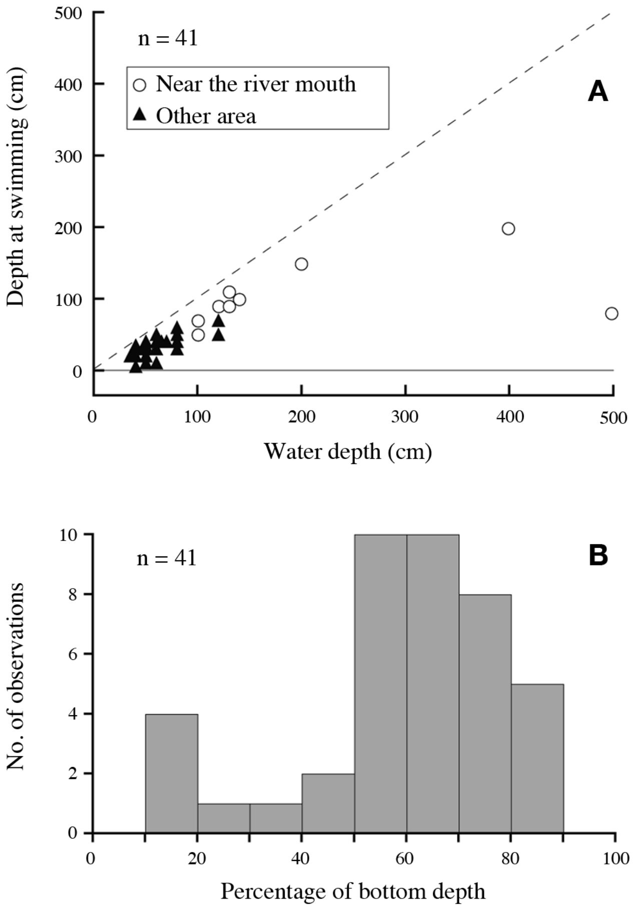

Figure 3. – **A**: Relationships between water depth and depth of swimming of newly recruited *Sicyopterus japonicus* post-larvae observed in the estuary of the Ota River from April to July 2008. Dashed and thin lines indicate bottom depth and swimming depth at 0, and open circles and closed triangles indicate observations near the river mouth and other areas, respectively. **B**: Percentage of bottom depth with the surface as 0 where post-larvae were swimming.

The post-larvae of *S. japonicus* were usually observed near the river banks where there were slow water flows with vegetation and rocks and they were not observed in the centre of the flow. Though water velocity was measured only once, the general trend of fast flow in the centre and slow flow near the banks was similar throughout the observations. Many post-larvae were observed in the middle layers or from the bottom to middle in areas shallower than 150 cm (Fig. 3A). Although the number of observations was only a few, larvae were also observed in middle and upper surface layers in areas with water depths of 200-400 cm near the river mouth (Fig. 3A). Regarding their swimming depth compared to the water depth (with the surface as 0), the majority of the larvae were observed in the middle layers, at 50-70% of the water column depth (Fig. 3B). There was no clear relationship between number of individuals in a shoal and swimming depth (not shown). They were observed to slowly swim upstream and sometimes stop (Fig. 1A). When they stopped, they always displayed a head-down stationary holding (hovering) behaviour (Fig. 1B), as previously reported (Iida *et al*., 2015).

DISCUSSION

Our observations in the lower reaches of the Ota River revealed that the shoals of *S. japonicus* were located in the middle layer throughout the observation area, and the upper half of the water column near the river mouth where the river is deeper than in the upper reaches, and in the lower half of the water column in the shallower upper reaches. The adult *S. japonicus* are benthic and many juveniles slightly larger than recruiting post-larvae were observed already settled on the bottom in the middle reaches of the river at 3.1 km from the river mouth, and they were observed shoaling on the bottom and sometimes swimming off the bottom before becoming solitary individuals (Iida, 2010; Iida, pers. obs.). However, post-larvae in the estuary were not seen on the bottom. According to Pinet (2014), the observation area is a partially mixed estuary, and stratification was often seen during the observations. Their swimming depth within the middle layer of the water column might indicate that the post-larvae simply swim in the water column composed of seawater rather than surface freshwater near the river mouth, as they recruit with flood tides (Iida *et al*., 2015). Another reason explaining the post-larvae swimming depth within the middle layer may be to avoid estuarine benthic predators.

During the underwater observations, many shoals of *S. japonicus* post-larvae were observed. Shoaling is a typical predator avoidance tactic used by many types of fish (Pitcher and Parrish, 1993). Adult gobies *Tridentiger* sp. resting on the bottom were sometimes observed to attack *S. japonicus* and other gobioid post-larvae (*Gymnogobius* spp., and *Rhinogobius* spp.) passing near them (Iida *et al*., 2015; Iida, pers. obs.). The shoaling behaviour of the post-larvae would seemingly be effective against these types of predator attacks. As previously reported, recruiting post-larvae of *S. japonicus* show a head-down stationary holding (hovering) behaviour when they stop swimming (Iida *et al*., 2015). This may also be related to predator avoidance due to their appearance looking like other objects such as broken pieces of seagrass or leaves as discussed in Iida *et al*. (2015).

S. japonicus post-larvae were observed near the river bank. This location differed from where the newly recruited juveniles of another sympatric amphidromous fish, the ayu, *Plecoglossus altivelis altivelis* (Osmeriformes) were observed in the Ota River during this study. The juveniles of *P. a. altivelis* were usually observed in shoals in the centre of flow. Though *P. a. altivelis* has an amphidromous life cycle similar to *S. japonicus*, they migrate back to freshwater as juveniles. The size of juvenile ayu at the centre of flow was reported to be 20–50 mm SL in the Shimanoto River in Kochi, Japan (Takahashi, 2005), which is larger than most *S. japonicus* post-larvae (approximately 27 mm SL: Iida *et al*., 2015). Thus they could have better swimming ability in comparison with the *S. japonicus* post-larvae at recruitment. Although it was not observed in the study site, it has been reported that newly hatched larvae of *P. a. altivelis* stay in the estuary and in the river mouth not only just after downstream migration, but also they can use those areas as a nursery habitat in large rivers (Takahashi, 2005). In such cases, small larvae stayed near river banks where water flow is moderated while large juveniles swam in the centre of flow (Takahashi, 2005). It is possible that *S. japonicus* post-larvae swim near the river bank because of their poor swimming ability just after recruitment from the sea.

The post-larval recruitment from the sea to rivers occurs simultaneously with a metamorphosis consisting of drastic morphological and physiological changes that allow the newly recruited *S. japonicus* post-larvae to osmoregulate in their new freshwater environment. They also have to change their lifestyle from planktonic to benthic, and their diet from plankton to benthic algae, as reported in previous studies (Dôtu and Mito, 1955; Shen and Tzeng, 2002; Iida *et al*., 2015; Sahara *et al*., 2016). In this context, this study indicates that the post-larvae mostly use the mid-range depths near the shorelines as they move through the estuary while they acclimate to the habitat changes. Further detailed studies can increase the understanding of the behavioural ecology of *S. japonicus* post-larvae as they pass through the estuary and adapt to their later life in freshwater.

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